Antidiabetic activities of *Syzygium hemisphericum* (Wight) Alston and *Syzygium kanarense* (Talbot) Raizada

Ph.D. Final Synopsis

Submitted to

The Registrar (Evaluation)

Mangalore University

Mangalore 574199

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Registration number: MU/EXB/Ph.D./CR.80/A.B./2019-20/E.13

Title: Antidiabetic activities of *Syzygium hemisphericum* (Wight) Alston and *Syzygium kanarense* (Talbot) Raizada

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Chapter One

General Introduction

Introduction

Diabetes mellitus is a metabolic disease characterized by raised blood glucose levels. The World Health Organisation (WHO) reports that globally 422 million are affected by diabetes annually and is the sixth leading cause of mortality. Diabetes-related deaths increased by 125.5 percent between 1990 and 2017, with India leading the 195 nations and territories evaluated (Lin et al., 2020). Hyperglycemia is caused by a lack of insulin synthesis and action or both. There is a need to discover newer therapeutic approaches to reduce long-term diabetic consequences. Herbal medicines are often preferred to treat both acute and chronic disorders due to their efficacy, lesser side effects, and affordability. There are a variety of Indian medicinal plants with anti-diabetic characteristics and other beneficial properties, as well as manufactured herbal medications in the market (Prakash et al., 2017).

Syzygium (Myrtaceae) is one of the largest angiosperm genera, with 1200 species. India has 102 species of *Syzygium* of which 44 are endemic (Shareef & Santhosh, 2020). *Syzygium* spp. have long been used as medicine in many parts of the world, having proven medicinal benefits for a variety of maladies. Ayurveda, Homeopathy, and other folk remedies use herbal formulations of different species of *Syzygium* to treat a variety of acute and chronic ailments (Cock & Cheesman).

Many species of *Syzygium* have been studied for their anti-diabetic properties. The bark of *Syzygium calophyllifolium*, *Syzygium samarangense*, and *Syzygium cumini* have traditionally been used as anti-diabetic (Ayyanar et al., 2012; Chandran et al., 2017). In Ayurveda, Unani, and various folk medicine systems, *Syzygium cumini* is a frequently used antidiabetic plant (Akbar, 2020). There is evidence that *Syzygium cumini* mother tincture, which is used in homeopathic diabetes treatment, has remedial effect on carbohydrate and lipid disorders (Boericke, 2002). *Syzygium* spp. are rich in essential oils and other secondary metabolites like phenolics, terpenes, flavonoids, anthocyanins, tannins, etc. Crude extracts and isolated bioactive components like myricetin, catechin, and gallic acid from various *Syzygium* species have promising anti-diabetic effect (Kasetti et al., 2012; Nallappan et al., 2023).

Syzygium hemisphericum (Wight) Alston is a less-known species of the Western Ghats with known medicinal potential in the traditional system. *Syzygium kanarense* (Talbot) Raizada is a recently rediscovered species having a very limited distribution in India's Western Ghats. Studies on these plants are carried out on the following objectives.

Objectives

- To study *in vitro* antioxidant and antidiabetic properties of *Syzygium hemisphericum* and *Syzygium kanarense* extracts.
- To evaluate the antidiabetic activity of the extracts in STZ+ NA-induced diabetic rats.
- Phytochemical profiling of the extracts using GC-MS and HR-LC-MS analysis.
- Synthesis, characterization, and pharmacological evaluation of zinc oxide nanoparticles using bark aqueous extracts.

Chapter Two

In vitro antioxidant and *in vitro* antidiabetic activities of *Syzygium hemisphericum* and *Syzygium kanarense* bark and leaf extracts

Introduction

Plant constituents are divided into primary and secondary metabolites based on their function. The secondary metabolites found in plants are of therapeutic value. *Syzygium* spp. are rich in essential oils and other secondary metabolites like phenolics, terpenes, flavonoids, anthocyanins, tannins, etc. Phytochemical studies on seeds of *Syzygium cumini* revealed the presence of biologically active components in ethyl acetate and methanol extracts (Kumar *et al.,* 2009). *Syzygium* spp. are well-known for their antioxidant and antidiabetic activity. The leaf methanol and aqueous extracts of *S. samarangense* and *S. aqueum* exhibited very good antioxidant activity. Seed methanol extract of *S. cumini* has an excellent α- amylase inhibitory activity (Aung *et al*., 2020). In this chapter, phytochemical screening, *in vitro* antioxidant and antidiabetic properties of methanol and aqueous extracts of *Syzygium hemisphericum* leaf (SHLM, SHLW), bark (SHBM, SHBW); *Syzygium kanarense* leaf (SKLM, SKLW) and bark (SKBM, SKBW) extracts are discussed.

Materials and methods

Collection of plant materials

In the month of April, the leaves and stem bark of both the plants were collected from the Gerusoppa region of Uttara Kannada, Karnataka, India (12º48'525"N and 074º55'232"E). Freshly collected leaves and bark were cleaned, shade dried, powdered, and used further.

Preparation of plant extract

The powdered leaf and bark samples were subjected to soxhlation using methanol. Also, the dried powder was soaked in water for 48 hours in the water bath at 50ºC to obtain aqueous extract. The

extracts were dried in a rotary vacuum evaporator, weighed, and stored at 4ºC for further use. Each extract's yield was calculated and represented as percentage.

Preliminary phytochemical analysis and quantification

Following the established protocols (Raaman, 2006), the leaf and bark methanol and water extracts were examined for the presence of flavonoids, alkaloids, tannins, glycosides, terpenes, saponins, and steroids. Following the Folin-Ciocalteau method (Taga *et al*., 1984) the total phenolic concentration was determined and was expressed as gallic acid equivalents (GAE)/ gram of extract. The aluminium chloride method (Zhishen *et al*., 1999) was used for flavonoid quantification and was expressed as quercetin equivalents (QE)/ gram of extract. All the experiments were performed in triplicates. The tannins in plant extracts are measured using Folin- Denis (FD) method (Bajaj & Devasharma, 1977) and the amount of tannin was calculated in tannic acid equivalents (TAE) /g of extract.

Antioxidant assays

Using DPPH assay (Banerjee *et al*., 2005) and the reducing power assay (Gonzalez- Palma *et al., 2016)* the antioxidant potential of all the extracts was assessed.

***In vitro* antidiabetic activity**

Using α-Amylase inhibitory assay (Bernfeld *et al.,* 1995), α-glucosidase inhibitory assay (Al-Hajj *et al.,*2016) and *In vitro* glucose uptake by isolated rat hemidiaphragm (Sreeranjit & Lal, 2003) the antidiabetic potential of all extracts were assessed.

Statistical analysis

The data were statistically analysed using GraphPad Prism software (Version *5.03*). One-way ANOVA followed by Newman-Keuls Multiple Comparison Test were carried out. The results were expressed as Mean ±SD.

Results

Preliminary phytochemical analysis and quantification

All the extracts contained flavonoids, tannins, saponins, and glycosides. Terpenes were present in all the extracts except in SKBW and SHBW. The total phenolic content was highest in SKBM (345.81±7.21 mg GAE/g) (**Figure 1A**). The SKBW extract had 199.07± 5.92 mg QE/g flavonoid which is higher than other extracts (**Figure 1B**). In contrast with leaf extracts, bark extracts of both plants contain a higher level of flavonoids. The result of tannin estimation is given in **Figure 1C**. Aqueous extract of *Syzygium kanarense* bark had the highest (155.51± 4.31mg TAE/g) tannin content and leaf methanol extract (79.41±1.91 mg TAE/g) showed the least content.

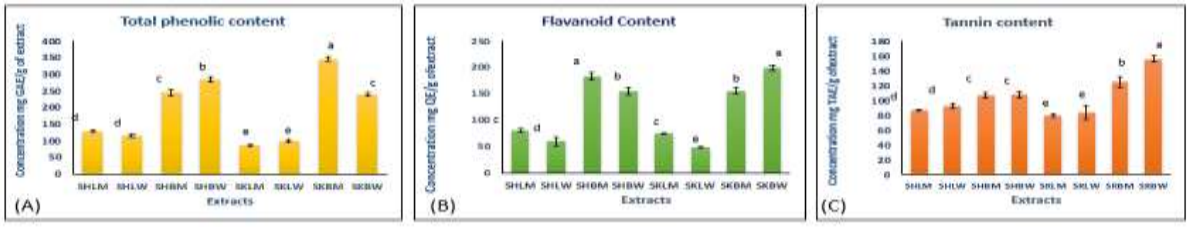

Figure 1. Total phenol (A), flavonoid (B) and tannin (C) content in the extracts. Values are the mean (n=3) ± Standard deviation. The different alphabets indicate the significant difference ($p<0.05$) between the means.

Antioxidant potential of plant extracts

All the extracts showed significant DPPH radical scavenging activity (**Figure 2**). The results showed that bark extracts have higher DPPH radical scavenging activity compared to leaf extracts.

The reducing power of extracts is given in **Figure 3**. The higher the absorbance the greater the antioxidant activity. All samples have concentration-dependent reducing powers. SKBW (1.745± 0.032 at 100 µg/ml) had the highest absorbance.

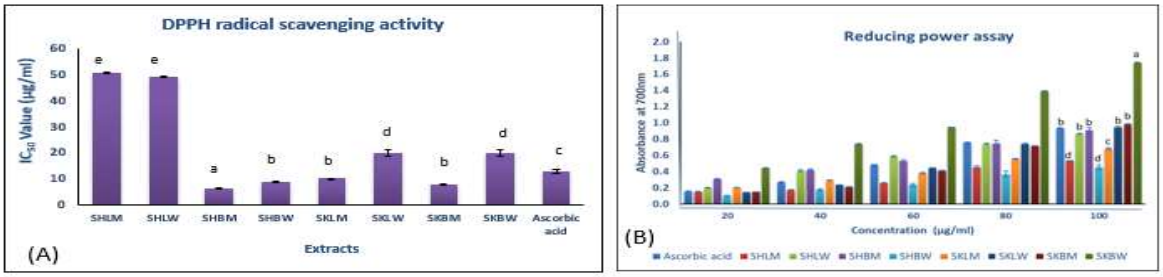

Figure 2. DPPH radical scavenging activity (A) and reducing power assay (B). Values are the mean (n=3) ± Standard deviation. The different alphabets indicate the significant difference ($p<0.05$) between the means.

In *vitro* antidiabetic activity

Figure 3A reveals the α-amylase IC_{50} values of all the extracts and acarbose. The IC_{50} of bark extracts was highly significant ($p<0.05$) when compared with the leaf extracts. The findings in **Figure 3B** show that SKBM had the greatest α-glucosidase inhibitory activity among the eight extracts. The least activity was seen with leaf extracts. All tested extracts (10mg/ml) enhanced the glucose uptake (**Figure 3C)** and were found to be maximum in bark extracts. Additionally, when insulin was present, SHBW and SKBW had a more significant effect in comparison to only insulin.

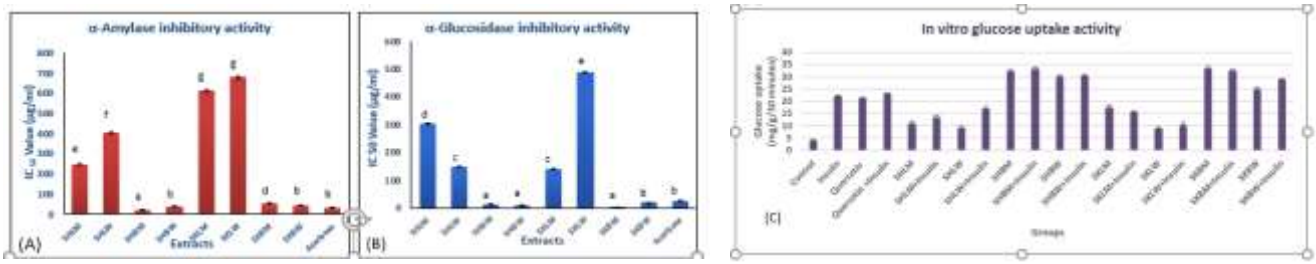

Figure 3. Effect of extracts on *In vitro* antidiabetic activity. (A) α-amylase, **(B)** α-glucosidase and **(C)** glucose uptake by isolated rat hemidiaphragm. Values are the mean (n=3) ± Standard deviation.

Chapter Three

Antidiabetic potential of *Syzygium hemisphericum* and *Syzygium kanarense* bark extracts in streptozotocin + nicotinamide (STZ+ NA) induced diabetic rats

Introduction

Diabetes is a metabolic disease characterized by hyperglycemia as a result of defects in insulin secretion or insulin action. A lack of insulin disrupts the metabolism of proteins, fats, and carbohydrates, resulting in persistent hyperglycemia. As the condition worsens, serious consequences from diabetes such as retinopathy, nephropathy, cardiovascular problems, and microbial infections start to appear.

Diabetes is mostly treated with insulin and oral hypoglycemic medications, while antidiabetic medicinal herbs are being used in Indian traditional medicine. Many authors have evaluated the potentiality of *Syzygium* plant extracts and purified compounds in treating diabetes and diabetes-related diseases Three decades before insulin was discovered, it was claimed that the fruits of *Syzygium* sp. were used to reduce blood glucose levels in hyperglycemia (Helmstadter 2008).

Streptozotocin- nicotinamide (STZ- NA) administered diabetic rat model is the preferred model to examine the antihyperglycemic action of various drugs in type 2 diabetes. This model is experimentally amenable since it maintains mild hyperglycemia and moderately decreased β-cell mass with medium insulin secretion (Novelli *et al*., 2001)

Methodology

Ethical approval

Following the guidelines of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), the Government of India, SDM Centre for Research in Ayurveda & Allied Sciences, SDM College of Ayurveda Campus, Udupi, Karnataka, India has approved the protocol (Ref.No: SDMCRA/IAEC/MU-09 dated 07/01/2019).

Healthy, adult male albino Wistar rats weighing between 180-220 g were selected for the experiments. Animals were maintained with a standard pellet diet and water *ad libitum*, were housed in polypropylene cages maintaining a temperature between 22-25 ºC, relative humidity between 50-60%, and 12-12 hours of light-dark cycle.

Acute oral toxicity study

Following the guidelines of Organisation for Economic Co-operation and Development- 425 (OECD-425), the acute oral toxicity test was carried out. A test dose of 2000 mg/kg was administered orally to the rats as per the 'Principle of the Limit Test' of OECD. Overnight fasted male rats (n=5) were fed 2000 mg/kg of SHBM, SHBW, SKBM and SKBW extracts. The rats were closely monitored for 4 hours for any behavioral, autonomic, and neurological reactions. Further, rats were observed every 24 h for mortality throughout the entire study period (i.e.,14 days).

Oral glucose tolerance test (OGTT)

Normal male Wistar rats (n=4) were used for this study. The overnight fasted rats were orally administered with one dose of glibenclamide (5 mg/kg), 300 mg/kg and 600 mg/kg of SHBM, SHBW, SKBM and SKBW respectively. After 30 min, 2000 mg/kg glucose was fed orally and the blood glucose levels were measured at 0, 30, 60, 120,180, and 240 minutes to determine glucose tolerance in various groups. Blood sample from the tail vein was tested using Accu-Chek Active glucometer.

Assessment of antihyperglycemic activity

In overnight fasted animals diabetes was induced. They were administered (i.p) with 110 mg/kg of nicotinamide (NA). 15 minutes later 60 mg/kg of streptozotocin (STZ) was given by a single intraperitoneal injection. The solvent for STZ was 0.1M citrate buffer (pH 4.5) and normal saline for NA. Elevated blood sugar levels after 72 hours of induction confirmed hyperglycemia. Animals having a blood glucose level above 300 mg/dl were employed for the study.

The experimental rats were allotted into eleven groups. Each group had six animals.

Group I: Normal control rats (Saline 5 ml/ kg)

Group II: Diabetic control rats (60 mg/kg of Streptozotocin + 110 mg/kg of Nicotinamide)

Group III: Diabetic + Glibenclamide (5 mg/kg)

Group IV: Diabetic + SHBM (300 mg/kg)

Group V: Diabetic + SHBM (600 mg/kg)

Group VI: Diabetic + SHBW (300 mg/kg)

Group VII: Diabetic + SHBW (600 mg/kg)

Group VIII: Diabetic + SKBM (300 mg/kg)

Group IX: Diabetic + SKBM (600 mg/kg)

Group X: Diabetic + SKBW (300 mg/kg)

Group XI: Diabetic + SKBW (600 mg/kg)

The diabetic rats were administered with the standard drug and bark extracts orally as given in the above dosage for 21 days to assess their antihyperglycemic activity. The blood glucose level was recorded on the 5^{th}, 10^{th}, 15^{th}, and 21^{st} day with Accu-Chek Active glucometer. The change in body weight of the rats was monitored in a digital weighing balance. Animals were sacrificed by cervical dislocation. Pancreas, liver, and kidney tissue samples were washed with normal saline and stored in phosphate buffer saline (pH 7.4) for further studies.

Assessment of biochemical parameters in serum

On the 21^{st} day, the blood samples were collected by a retro-orbital puncture from the anesthetized overnight fasting rats. Glycosylated haemoglobin (HbA1c) was measured in blood. The total protein (TP), albumin (Alb), urea, creatinine, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL- C), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the serum were measured using a commercial kit (Agappe diagnostics ltd, India). Using Friedewald's equation the concentration of very low-density lipoprotein cholesterol (VLDL- C), and low-density lipoprotein cholesterol (LDL- C) were calculated. Liver glycogen content and the activity of glucose 6-phosphatase and fructose 1, 6-bisphosphatase, and catalase were measured.

Histopathology

The liver, pancreas, and kidney tissues were fixed with 10% buffered formalin, sliced, embedded in paraffin blocks, sectioned using a microtome (≈5µm), and stained with haematoxylin and eosin. The sections were observed under 40X using a Nikon Optiphot-2 microscope with Nikon Digital Sight (DS-F1), Japan.

Results

Acute oral toxicity study

The treated animals did not show any significant physical or behavioural changes in the initial 4 hours. Motor activity, food and water intake were unaffected. Additionally, there was no salivation, aggression, diarrhoea and skin irritations in the 14 consecutive days of observation. There was no mortality at fixed dosage of 2000 mg/kg body weight.

Oral glucose tolerance test (OGTT)

The oral administration of the extracts enhanced glucose tolerance in oral glucose-loaded rats. After an initial spike, the glucose level was significantly reduced. Compared to the extract-treated groups, sudden hypoglycaemia was observed in the glibenclamide-treated group at 30 min (98.33±4.22 mg/dL). Both the lower (300 mg/kg) and higher (600 mg/kg) doses of both plant extracts bring blood glucose levels to normal after 180 minutes, indicating the extracts' potential to provide glucose tolerance. The extracts bring about a steady and gradual decrease in the glucose level in comparison to the glibenclamide-treated group.

Assessment of antihyperglycemic activity

Both the extracts showed significant antihyperglycemic activity throughout the experimental period. In comparison to the other groups, 600 mg/kg dosage of extracts were found to be more effective in lowering blood glucose levels ($p < 0.001$). The extracts induced weight gain during the study period.

Effect on biochemical parameters

The level of oxidative stress marker enzymes AST and ALT were significantly ($p<0.05$) lower compared to the diabetic control group. Also, a significant ($p<0.001$) enhanced catalase activity was observed in extract-treated groups. The albumin (Alb) and total protein (TP) levels in the serum were comparable to the normal rats. There was a significant ($p<0.05$) decrease in creatinine and urea levels; and glucose 6-phosphatase and fructose 1, 6-bisphosphatase activity in the extract-treated groups.

Effect on lipid profile

Diabetic rats treated with higher concentrations (600 mg/kg) of extracts showed similar effects as glibenclamide-treated rats. Similarly, when compared to the diabetic rats, a significant ($p<0.001$) decrease in TC, TG, LDL, and VLDL levels was noticeable in the extract (600 mg/kg) treated rats. Comparing diabetic rats to normal control rats, there was no difference in HDL levels. Liver glycogen level was comparable to the normal rats.

Histopathological studies

The diabetic rats treated with extracts showed improved liver architecture with normal hepatocytes arranged in cords around the central vein with mild dilation of sinusoids. The diabetic rats showed deformed glomeruli, degenerated Bowman’s capsule, and fat deposition in the medullary region. The normal control rats showed a normal kidney architecture with a narrow Bowman’s space and a clear demarcation of proximal and distal tubules without any fat deposition. The diabetic rats that received a high dose (600 mg/kg) of extracts showed glomeruli regeneration and had no fat

accumulation. A localized reduction in the size of pancreatic islet size and severe reduction in the beta cells were observed in STZ+ NA administered rats. Regeneration of islets and an increase in the number of beta cells were observed in diabetic rats treated with a lower dose (300 mg/kg) with restored acinar cells. The islets of Langerhans regenerated remarkably in diabetic rats receiving 600 mg/kg of the extract with numerous evenly distributed beta cells (**Figure 4**).

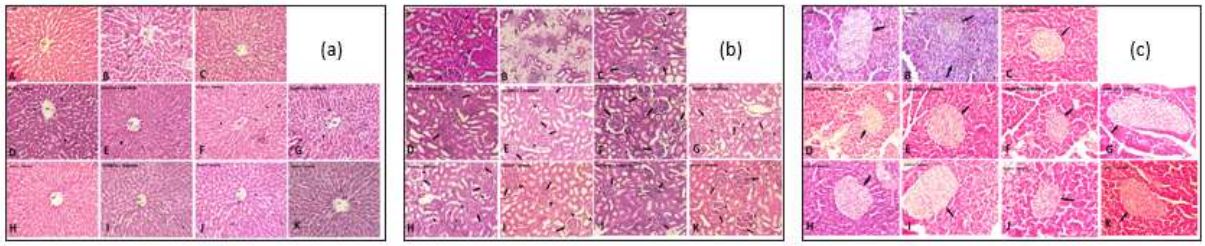

Figure 4. Effect of extracts on histopathology of (a) Liver, (b) Kidney and (c) Pancreas. Scale - 0.2 mm (A) Normal control, (B) Diabetic control, (C) Diabetic + Glibenclamide (5 mg/kg), (D) Diabetic + SHBM (300 mg/kg), (E) SHBM (600 mg/kg), (F) SHBW (300 mg/kg), (G) Diabetic + SHBW (600 mg/kg). (H) Diabetic + SKBM (300 mg/kg), (I) SKBM (600 mg/kg), (J) SKBW (300 mg/kg), (K) Diabetic + SKBW (600 mg/kg).

Chapter Four

GC- MS and HR-LC-MS based metabolite profiling of *Syzygium hemisphericum* and *Syzygium kanarense* bark extracts.

Introduction

Medicinal plants have been used for thousands of years to treat a wide range of diseases. The identification and standardization of phytoconstituents is necessary for improved therapy. The evidence-based reports are crucial in overcoming adverse health effects and drug development. In the present study, we investigated the phytoconstituents of crude extracts using GC-MS and HR-LC-MS approaches.

The largest flowering genus in the Myrtaceae family, *Syzygium*, has a long history of use in medicine with an array of biological activities. Many compounds, including volatile oils, flavonoids, tannins, saponins, glycosides, and phenols, are found in various parts of the plant with various medicinal effects. Many phytoconstituents with curative potency have been identified from several species of *Syzygium* using GC-MS and HR-LC-MS analysis. Compounds like 9-Octadecenamide, γ- sitosterols and methyl palmitate have proven pharmacological properties like hypolipidemic, antiulcer, anticancer, and antidiabetic (Mayasankaravalli *et al*., 2020) activities. Flavonoids like myricetin, rhamnoside, ellagic acid, catechin, quercetin etc. have antidiabetic,

antiproliferative and antimicrobial properties. Phenols and tannins like gallic acid, myrcitrin, caffeic acid, corilagin and coumaric acids have proved anti-inflammatory, antioxidant and antidiabetic activities (Uddin *et al*., 2022).

Materials and methods

Gas chromatography-mass spectroscopy (GC- MS) analysis

The phytoconstituents of SHBM and SHBM were studied using GC-MS (1300 GC -Thermo Scientific TSQ 8000) analysis. The oven temperature was gradually increased to 280 ºC, from 60ºC, at 5 ºC/min and the scan range varied from 50 – 700 m/z, with a suitable injection volume (1.0µl). Further, with the aid of the NIST library (open source), the phytochemical constituents were identified.

High resolution liquid chromatograph mass spectrophotometer (HR-LC-MS) analysis

HR-LC-MS analysis of SHBM, SHBW, SKBM and SKBW extracts were carried out on an Agilent HPLC system model- G6550A (MS Q-TOF) with 0.01% mass resolution. HR-LCMS was performed with an auxiliary speed of 100 µl/min, ejection speed of 100 µl/min, flush out factor of 5.0, and injection volume of 5µl. The mobile phase consisted of 0.1%formic acid in water and 90% acetonitrile + 0.1% formic acid in water.

Results

The most prevailing compounds in bark methanolic extract of *Syzygium hemisphericum* based on their peak area are 9- Octadecenamide (50.83%); 9, 12- Octadecadienoic acid (Z-Z), methyl ester (9.12%); 11-Octadecenoic acid, methyl ester (9.06%); Hexadecanoic acid, methyl ester (4.45%).

Based on peak area, 9-Octadecenamide (40.46%); 9-12 Octadecadienoic acid (Z-Z), methyl ester (9.60%); 11-Octadecenoic acid, methyl ester (9.04%); 1,8, 11- Heptadecatriene (6.24%); Glycidyl oleate (6.16%) and Hexadecanoic acid, methyl ester (4.55%) are the most abundant chemicals in the *Syzygium kanarense* bark methanol extract.

HR-LC-MS analysis confirmed the presence of many phytoconstituents belonging to major groups like polyphenols, terpenoids, lipids, and alkaloids.

Phenols like Gingerol, Shogaol, Resveratrol, and Cinnamic acid as well as flavonoids like Kanzonol Z and Kaempferol were detected in the bark methanol extract of *Syzygium hemisphericum.* The highest peaks were observed for a carotenoid Kaempferol 7-O-glucoside (23.33 min) followed by Gancaonin F (0.73 min).

The phytoconstituents detected in the bark aqueous extract of *Syzygium hemisphericum* are β-Sitosterol, Gallic acid, Hippophaenin A, Picein, Kaemferol, Cryptoflavin, [12]-gingerol with high detector counts.

The phytoconstituents detected in the bark methanol extract of *Syzygium kanarense* are 9 – Octacedenamide, 17-Hydroxylinolenic acid and Methyl 2,5-octadecadiynoate. The highest peaks were reported for Methyl 2,5-octadecadiynoate (23.32 min), 9 – Octacedenamide (22.56 min) followed by Gingerol (19.87 min).

The bioactive compounds such as Psammosilenin B, Ginsenoside Ro and D-Pantothenic acid. Methyl gallate, Gingerol and Glycyrrhizaisoflavone C, Centellasaponin C, Ginsenoside Ro, were detected in *Syzygium kanarense* bark methanol extracts.

Chapter Five

Synthesis, characterization, and pharmacological evaluation of zinc oxide nanoparticles using bark aqueous extract of *Syzygium hemisphericum* and *Syzygium kanarense*

Introduction

Metal oxide nanoparticles (MO nanoparticles) have found their vast applications in diverse fields like optics, catalysis, biomedical fields, and wastewater treatment in recent years. However, the "green approach" for the tailoring of the nanoparticles for specific applications is currently attracting attention worldwide. "Green chemistry" is an interdisciplinary area connecting life science and chemistry in the development of novel materials for the establishment of a sustainable environment (Zuin *et al*., 2021). The benefit of green synthesis is the possibility of the synthesis of desired featured nanoparticles for desired applications specifically in the biomedical field. The surface of the nanoparticles gets associated with the phytochemicals from the plant extract via "Dangling Bonds" which together enhance the properties of the final green nanoparticles.

One of the most common consequences of diabetes mellitus is foot ulcers or gangrene, which affects 15% of diabetics during their lifetime. Inadequate blood flow to the feet prolongs the healing process of wounds and can cause gangrene. Various green synthesized nanoparticles are now being used in wound infection cleaning (antimicrobial), to enhance collagen synthesis and as antioxidants (Younis *et al.,* 2022). Several studies have proven that plant extract-derived nanoparticles exhibit effective anticancer (Venugopal *et al*., 2017), antidiabetic, anti-inflammatory and antimicrobial (Chakravarty *et al.,* 2022) activity.

Materials and methods

Preparation of the extracts

The dried powder of *Syzygium hemisphericum* bark (SHB) and *Syzygium kanarense* bark (SKB) was soaked in water for 48 hours in the water bath at 50ºC to obtain an aqueous extract. The extracts were dried in a rotary vacuum evaporator, weighed, and stored at 4ºC for further use.

Synthesis of bark extract capped ZnO nanoparticles by solution-combustion synthesis method

The extract was magnetically stirred to obtain a homogenous solution. In a stoichiometric ratio, a known volume of homogenous extract solution was added to zinc nitrate hexahydrate crystals and the mixture was magnetically stirred for about 15 min. The mixture was then placed in a preheated (450 ± 5ºC) muffle furnace and maintained for 40 min. Then it was subjected to calcination at a temperature of 650°± 5°C for one hour.

Characterization of SHB@ZnO and SKB@ZnO nanoparticles

FTIR spectrometer (Shimadzu) analysis was carried out over a wide scanning range for the preliminary investigation of Zn-O bond formation. Diffraction spectra of the biogenic nanoparticles were affirmed from powder X-ray diffraction (p XRD) technique using Rigaku mini flex 600 (5th gen) with CuKα radiation. Field Emission Scanning Electron Microscope and Energy Dispersive X-ray spectroscopy (FESEM- EDX; Carl Zeiss, USA) were used for the analysis of microscopic surface traits and their elemental compositions. The optical characteristics were obtained from UV-Visible absorption spectroscopy (Shimadzu) from a wavelength range between 200-800 nm.

Antioxidant assays

Using DPPH assay (Banergee *et al*., 2005) the antioxidant potential of all the nanoparticles was assessed.

***In vitro* antidiabetic activity**

α- amylase, α-glucosidase inhibitory and *in vitro* glucose uptake assays were carried out by the methods given by Bernfeld *et al.,* (1995), Al-Hajj *et al*., (2016) and (Sreeranjit & Lal, 2003) respectively.

***In-vitro* anti-inflammatory activity**

The anti-inflammatory activity was determined using a human RBC membrane stabilization assay (Kouser *et al*. 2021).

Anticancer evaluation

A431 cancer cell lines have been used for this. The evaluation was done using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay (Saber *et al.*, 2018).

Antimicrobial activity

The antibacterial efficacy was evaluated against two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative bacterial strains (*Pseudomonas aeruginosa* and *Proteus*

vulgaris) by measuring the zone of inhibition (Mohamed *et al*. 2020) on the Muller – Hinton agar (MHA) plates. Standard antibiotic chloramphenicol (30 µg disc) was used as a positive control and the sterile distilled water was used as a negative control.

Results

Characterization of SHB@ZnO and SKB@ZnO nanoparticles

FT-IR analysis

The formation of ZnO nanoparticles can be affirmed by comparing the FT-IR spectra of the extract and extract derived nanoparticles ranging from 400 to 4000 cm^{-1}. The broad peak at 3280 cm^{-1} indicates the stretching frequency of the – OH group of polyphenols present in the crude extract. The peaks around 2346 cm^{-1}, 2234 cm^{-1} represent -C≡C and –C≡N stretching respectively. The prominent peaks at 411 cm^{-1} and 398 cm^{-1} correspond to the formation of the Zn-O bond (**Figure 5**).

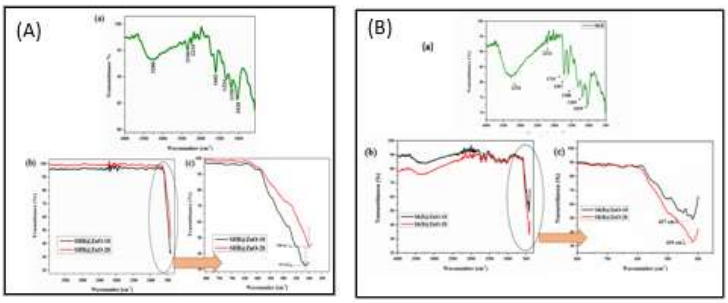

Figure 5. FTIR spectra of (A) *Syzygium hemisphercum* and (B) *Syzygium kanarense* (a) crude extract (b,c) ZnO nanoparticles

Diffraction studies by pXRD analysis

The ZnO nanoparticles showed cubic crystalline structures which were affirmed by p-XRD patterns. The patterns obtained evidenced the synthesis phase purity of crystalline nanoparticles. The peaks corresponding to the single phase were confirmed and other additional peaks were absent. The crystallite size was evaluated for all the indexed planes and the average crystallite size was found to be in the range of 10 nm with a large specific surface area.

FESEM-EDS analysis nanoparticles

The surface morphology of the ZnO nanoparticles was evaluated by the Field Emission Scanning Electron Microscopy technique. The nanoparticles were analyzed at different magnifications (1 µm, 200 nm, and 100 nm). Aggregated dumbbell-shaped morphology at higher magnification was observed at nanoparticles derived from SHB aqueous extract.

Energy-dispersive spectroscopic (EDS) analysis

The purity of the nanoparticles is ascertained by Energy Dispersive Spectroscopic techniques.

Using the spectral data, the presence of Zinc (Zn), Oxygen (O), and Carbon (C) is revealed.

UV-Visible Analysis

The obtained peaks are in the range of 200 to 300 nm and 357 nm. These peaks correspond to the presence of numerous water-soluble polyphenols in the bark extracts. The UV-Vis spectrum obtained for the extract-derived ZnO nanoparticles shows several peaks characteristic of the formation of ultrafine nanoparticles. The smaller peaks give evidence for the surface-attached bioactive molecules to the ZnO nanoparticles.

***In vitro* antioxidant study**

The results show impressive DPPH free radical scavenging capability in a dose-dependent manner. From the slope of the plot between the concentration and the % inhibition, the IC_{50} values were determined and were found to be 236.74 ±2.55 µg/mL for SHB@ZnO nanoparticles and 312.61 ±3.91µg/mL for SKB@ZnO nanoparticles.

***In vitro* anti-diabetic study**

The α-amylase and α-glucosidase IC_{50} for the SHB@ZnO were 77.39±0.24 µg/mL and 68.35 ±2.43 µg/mL respectively. It is evident from the findings that SHB@ZnO has considerable antidiabetic activity compared to the SKB@ZnO nanoparticles.

***In vitro* anti-cancer study**

The nanoparticles have an impressive action towards A431 cancer cell lines. The IC_{50} value of the MTT assay was as low for the extract-derived ZnO nanoparticles compared to that for the crude extract.

***In-vitro* anti-inflammatory activity**

The study revealed the dose-dependent action of nanoparticles and the aqueous extracts and stabilizing the RBCs i.e., with the increase in the sample concentration (50 -250 µg/mL). The efficiency obtained is compared with the standard diclofenac sodium which has a stabilization percentage ranging from 35.80 to 80.60 %.

Antimicrobial assay

The concentrations were varied (25 µg to 100 µg) to get an appreciable zone of inhibition and to know the optimum concentration of extract and nanoparticles as antibacterial agents. The zone of inhibition increases with an increase in concentration. The results were compared with the standard Chloramphenicol (30 µg). The aqueous extract of *Syzygium hemisphericum* showed antimicrobial efficacy only for *Proteus vulgaris.* The study implied that the SKB@ZnO nanoparticles are more potent antibacterial agents at a concentration of 100 µg for strains of *Staphylococcus aureus* (6.33±0.4 mm).

Highlights of the study

- Worldwide, diabetes is the major killer disease and the antidiabetic drugs which are in use cause many side effects. Traditionally, some of the *Syzygium* spp. are in use for treating diabetes, and many species of *Syzygium* are being assessed for their antidiabetic property. This study aims to assess the antidiabetic effectiveness of *Syzygium hemisphericum* and *Syzygium kanarense*.
- Bark extracts showed the strongest α –glucosidase and α –amylase inhibitory activity in contrast to the leaf extracts. Antioxidants, phytoconstituents, and antidiabetic action, as well as protection against free radical damage, were found to be significantly correlated.
- Administration of bark extracts to STZ-NA-induced diabetic rats at 300 mg/kg and 600 mg/kg orally for 21 days exhibited statistically significant ($P < 0.001$) and dose-related drop in blood sugar levels, serum lipid, and hepatorenal parameters.
- The extract-treated rats showed rejuvenated islets and increased β-cell density in the pancreas, improved liver architecture, and glomerular regeneration without fat deposition. The study suggests that both methanol and aqueous extracts of bark exhibit dose-dependent antihyperglycemic activity.
- The bark of both plants contains various valuable secondary compounds which have various medicinal properties that can be useful for the treatment of various diseases.
- Both plants are excellent sources for the synthesis of nanoparticles (<10nm). ZnO nanoparticles showed excellent antioxidant, anti-inflammatory, antidiabetic, anticancer and antibacterial activities. The study can pave the way for the synthesis of environmentally -friendly, cost-effective ZnO nanoparticles from medicinal plants.

List of publications

Sushmitha, C. H., & Krishnakumar, G. (2023). Evaluation of antidiabetic potential of *Syzygium kanarense* (Talbot) Raizada in streptozotocin-nicotinamide induced diabetic rats. *Plant Science Today*, *11*(1), 54-63.

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Sushmitha, C. H., & Krishnakumar, G. (2021). Antioxidant and antidiabetic potential of *Syzygium kanarense* (Talbot) Raizada (Myrtaceae) - an endemic species of the Western Ghats, India. International conference on new horizon in plant science (NHPS 2020). Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India. 4^{th} - 9^{th} January 2021. 132-133. (ISBN 978-81-940888-3-7).

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